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Title: Comparative validity of vitamin C and carotenoids as indicators of fruit and vegetable intake: a systematic review and meta-analysis of randomised controlled trials

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Abstract

Circulating vitamin C and carotenoids are used as biomarkers of fruit and vegetable intake in research, but their comparative validity has never been meta-analysed. PubMed, EMBASE, CENTRAL, CINAHL and Web of Science were systematically searched to December 2013 for randomised trials of different amounts of fruit and vegetable provision on changes in blood concentrations of carotenoids or vitamin C. Reporting followed PRISMA guidelines. Evidence quality was assessed using the GRADE system. Random effects meta-analysis combined estimates and meta-regression tested for sub-group differences. Nineteen fruit and vegetable trials (n=1382) measured at least one biomarker, of which nine (n=667) included five common carotenoids and vitamin C. Evidence quality was low and between-trial heterogeneity (I^2) ranged from 74% for vitamin C to 94% for α-carotene. Groups provided with more fruit and vegetables had increased blood concentrations of vitamin C, α-carotene, β-carotene, β-cryptoxanthin, and lutein but not lycopene. However, no clear dose-response effect was observed. Vitamin C showed the largest between group difference in standardised mean change from pre- to post-intervention (0.94, 95% CI 0.66, 1.22), followed by lutein (0.70, 95% CI 0.37, 1.03) and α-carotene (0.63, 95% CI 0.25, 1.01) but all confidence intervals were overlapping suggesting no biomarker responded more than others. Therefore, until further evidence identifies a particular biomarker to be superior, group-level compliance to fruit and vegetable interventions can be indicated equally well by vitamin C or a range of carotenoids. High heterogeneity and a lack of dose-response suggest that individual-level biomarker responses to fruit and vegetables are highly variable.

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Introduction

Higher fruit and vegetable intake has been associated with reduced risk of cardiovascular disease (CVD), all-cause mortality and specific types of cancer [1; 2; 3; 4]. The World Health Organisation (WHO) recommend 400g of fruit or vegetables per day [5], equating to five 80g portions, and encourages the evaluation of interventions to increase intake of fruits and vegetables [5]. Adherence to advice in dietary interventions is frequently assessed by self-report tools [6], which have known limitations [7; 8; 9]. Social approval bias specifically occurs in fruit and vegetable interventions resulting in overestimated self-reported intakes [9]. Objective measures of fruit and vegetable intake are therefore essential to improve confidence in research findings.

Blood-based biomarkers, resulting from the metabolism of fruits and vegetables in the body, have been proposed as objective indicators of fruit and vegetable intake [10]. Biomarkers correlate weakly with fruit and vegetable intake assessed by a range of self-report tools [11; 12]. For example, a meta-analysis estimated the correlation between dietary and plasma vitamin C to be just r=0.3 [13]. However, comparing biomarkers with self-reported intakes to establish validity is flawed because true intakes are poorly represented by self-report tools. Dietary randomised controlled trials (RCTs), with direct observation or provision of different amounts of fruit and vegetables to different groups, provide a more robust way to validate biomarkers of changes in dietary intake. Randomisation may rule out confounding from other lifestyle factors and the direct observation or provision of fruit and vegetables may allow true intakes to be more accurately estimated compared with self-reported intakes from groups randomised to different dietary advice (potential for differential priming for social desirability bias).

In a systematic review of RCTs published up to April 2009 [14] the most commonly measured and consistently responsive biomarkers for fruits and vegetables were carotenoids and vitamin C. However, there was no meta-analysis to quantify the responsiveness or examine the consistency of response of carotenoids and vitamin C. Furthermore, there was no comparative analysis of different biomarkers measured within the same set of studies, which would allow the relative validity of different biomarkers to be established. The current systematic review updates the existing review with a specific focus on the effect of changes in

fruit and vegetable intake on blood concentrations of vitamin C and carotenoids in RCTs with food intake directly observed or provided to participants. To provide a direct comparison of different biomarkers, our primary analysis focussed on those trials in which a common set of vitamin C and five carotenoids were measured.

Methods

The review was reported according to items in the PRISMA statement (**Supplementary table 1**).

Trial identification

A previous systematic review provided studies prior to 2009 in the current review [14]. Updated searches were conducted (by LJ) from April 2009 (last search date of previous systematic review [14]) to December 2013 in PubMed, EMBASE, CENTRAL, CINAHL and Web of Science using terms related to fruits and vegetables, dietary intervention studies and biomarkers (see online supplementary information for detailed search strategy). Any relevant systematic reviews were obtained and their reference lists were examined for additional references. Citations were screened by one reviewer (MP or LJ) and hard copies of relevant articles obtained. These were screened by one reviewer (MP) and checked for inclusion by a second reviewer (LJ).

Inclusion and exclusion criteria

Randomised controlled trials of different amounts of fruit and vegetable intake (where some food intake was observed or provided) with outcomes of plasma or serum vitamin C or carotenoids were included in the review. Interventions of any duration were considered for inclusion. Trials altering other aspects of diet, in addition to fruit and vegetable intake, for example low-fat diets, were excluded to avoid the possibility that changes in blood-based biomarkers were a result of dietary changes other than fruit and vegetables. Intervention studies of a single fruit or vegetable were excluded. Findings from these types of interventions may underestimate the utility of biomarkers for measures of general fruit and vegetable intake as any single food contains a more limited range of nutrients. Trials where fruit and vegetable

intake was encouraged through dietary advice were excluded since adherence to the advice is harder to estimate. Trials in healthy or unhealthy populations were included, including populations with high CVD risk factors or impaired glucose metabolism. However, trials in populations with abnormalities in micronutrient metabolism or vitamin deficient populations were excluded. Trials were included if they reported biomarker measurements, either as changes from baseline or as baseline and post-intervention values, and if information was available on the amount of fruit and vegetables consumed in each intervention group.

Data extraction

Data on trial and population characteristics and outcomes were extracted into an Excel form that was piloted on a sample of trials before use (by MP, MS, LJ, CM). Data extracted on trial characteristics included the type of trial (parallel or crossover), duration of intervention, information on the duration of pre- and within-intervention washout periods, the amount and types of fruits and vegetables consumed and the mode of administration (some meals eaten under supervision vs. all meals at home), smoking status, fasting status at the time of biomarker measurement, the use of dietary supplements, inclusion and exclusion criteria and funding sources. Population characteristics included the sample size, country and type of sample, e.g. clinical or general population, and participant demographics, including age, sex and ethnicity were also extracted. Where available, data on baseline, post-treatment and change in biomarker concentrations were extracted for each trial arm. Where data on the amount of fruit and vegetables provided or biomarker levels was incomplete or lacked estimates of precision, authors were contacted. For four trials [15; 16; 17; 18], data were supplied by authors and included in the review.

Quality assessment

A risk of bias (ROB) assessment was conducted (by MP) using the Cochrane risk of bias tool [19]. Randomisation, allocation concealment, participant and assessor blinding, missing data, and selective outcome reporting were assessed. Other items hypothesised to potentially introduce risk of bias were also added: the exclusion of participants taking supplements or smoking, participant fasting at the time of blood sampling, diet adherence monitoring and sufficient intervention wash-out periods (for cross-over trials) (≥4 weeks). The ROB for each

trial was considered on the basis of whether any of the items, individually or in combination with others, were likely to have introduced bias and trials were assigned as having no, possible or high ROB. The overall quality of the evidence for each outcome was assessed with the GRADE system [20] that considers 1) the ROB across trials contributing to that outcome, 2) heterogeneity in the meta-analysis, 3) directness, or the generalisability of the population in the trial, 4) precision of the effect size and 5) risk of publication bias.

Data analysis

Standardised mean change (SMC) and standard deviation (SD) of biomarker concentrations from pre- to post-intervention were computed using the baseline SD within each trial arm, owing to variation in the units reported across studies (μmol/L; mg/dL; μmol/μmol of cholesterol; μmol/mol of lipid). Effect sizes (standardised mean difference (SMD)) were the difference of the SMC of biomarkers between arms with higher vs. lowest fruit and vegetable intake. The standard error of the SMD was computed from the variance of the SMC and the sample size in each arm. For trials with more than two arms, the arm with the lowest fruit and vegetable intake was compared against all other arms. To account for the use of the lowest intake arm in multiple comparisons, the sample size of that arm was divided by the number of comparison groups within that study [21]. Fruit and vegetable intake was described in terms of number of portions using standard UK portion sizes i.e. one portion equates to 80g of fruit or vegetables [22].

Mean differences in changes in biomarker between groups allocated different doses of fruits and vegetables across the whole study in crossover designs were assumed to be the same as mean differences between groups in parallel study designs. Where average biomarker concentrations pre- and post-intervention were described using medians or geometric means, these were assumed to approximate the mean; and 95% confidence intervals or interquartile ranges were transformed to approximate the SD assuming a normal distribution. Where data on change was not available, pre- and post-intervention mean (SD) concentrations were extracted and mean change was computed by subtracting pre-intervention mean from post-intervention mean in each arm. The SD of the standardised mean change was computed using standard equations [21] based on the SD at baseline and SD at follow-up within each

arm and biomarker-specific correlations (r) based on published associations between baseline and follow-up concentrations of biomarkers [23; 24]. Post-hoc sensitivity analyses were performed to check the influence of all assumptions on the results and the pattern of findings was unaltered.

For each biomarker, SMD (standard error (SE)) was pooled across all trials using random effects meta-analysis with inverse variance weights and heterogeneity was estimated using I^2 [25]. Heterogeneity of was considered low or high if I^2 was <25% or >75% respectively. For the primary analysis, data were combined for each biomarker for trials that included vitamin C, and a common set of 5 carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein and lycopene). Sub-group analyses planned a-priori were conducted for each biomarker using meta-regression to investigate potential dose-response effect (difference in fruit and vegetables intake between arms in each trial in g/day) and sources of heterogeneity, including differences by intervention duration (0-3 weeks vs. 4+ weeks, categories created based on data available); intervention compliance (meals observed vs. eaten at home); trial design (crossover vs. parallel); health status (healthy vs. unhealthy); location (Europe vs. US vs. Asia-Pacific); type of food provided (fruit and vegetables vs. vegetables only, categories created based on data available); baseline fruit and vegetable intake (<1 portion vs. 2-3 portions vs. 4-5 portions, categories created based on data available); fasting status (fasted vs. not); blood sample fraction (plasma vs. serum); risk of bias (low vs. possible vs. high); and sex (mixed vs. male vs. female). To check for a possible ceiling effect among participants with elevated biomarker concentrations, we also performed subgroup analyses by baseline biomarker concentrations (low vs. high based on median split, categories created based on data available). For sub-group analyses, all trials with that biomarker measured were used, regardless of the simultaneous measurement of other biomarkers. As substantial (I^2>75%) between-trial heterogeneity was observed, a post-hoc sensitivity analysis was conducted to examine the effect of excluding trials with outlying results (more than 2 standard deviations from the SMD) from the analysis. Statistical evidence of association was considered important at $p<0.05$. Data were analysed in Stata, version 12 (StataCorp, College Station, Texas).

Results

204

205 *Trial selection*

206 Of 3,759 unique records, 144 full text articles were assessed for inclusion and nineteen trials
207 were included in the review (**Figure 1**). Nineteen trials were identified in this review, 10 of
208 which were also included in the previous systematic review [14]. Out of the 19 trials, nine [23; 26;
209 27; 28; 29; 30; 31; 32; 33] assessed a common set of six biomarkers including five carotenoids and
210 vitamin C (**Supplementary table 2**) and were included in the comparative (primary) analysis.
211 Of the papers rejected on full text screening, the majority were excluded on the basis of the
212 intervention, often because trials involved only dietary advice, or because the intervention
213 targeted a single fruit or vegetable only. Other common reasons for exclusion were wrong
214 study design (not RCT with food provision) or wrong outcomes (no biomarker concentrations).

215

216 *Trial characteristics*

217 Trial characteristics for all the included trials are shown in **Table 1**. Twelve trials were
218 conducted in healthy populations [15; 17; 23; 27; 28; 31; 32; 34; 35; 36; 37; 38]. Two trials were conducted in
219 populations with increased CVD risk [29; 33] and single trials were in populations with obesity
220 [39], overweight [16], hypertension [30], elevated blood pressure [18] or chronic obstructive
221 pulmonary disease (COPD) [26]. Within trial differences in intake of fruit and vegetables
222 ranged from 2 to 13 portions /day. The sample sizes ranged from 20 to 246 participants
223 (median 64). For the nine trials included in the comparative analysis, the difference in amount
224 of fruit and vegetables between arms ranged from 2-7 portions/day.

225

226 *Quality of the evidence*

227 In the GRADE assessment of the quality of each outcome in the meta-analysis, no outcomes
228 were downgraded for imprecision or indirectness. However, most trials were considered to
229 have some ROB (**Figure 2**). Trials did not state that there was allocation concealment and
230 patient blinding was not possible. In a number of studies, there were inadequate pre- and
231 within-intervention washout periods and uncertainties around the true ingested amounts of
232 fruits and vegetables (less adherence monitoring) (**Figure 2**). In the absence of washout
233 periods, there was considered to be risk of pre-intervention or cross-treatment contamination.

In trials where consumption of fruit and vegetables was not directly observed, there was considered to be a likely over-estimation of the true ingested amount. A concern in some trials was the inclusion of participants using nutritional supplements, a lack of fasting at the time of outcome measurement and the inclusion of patients who smoked. Funnel plots suggested the possibility of publication bias and heterogeneity for α-carotene, β-carotene, β-cryptoxanthin, and vitamin C (based on the occurrence of studies outside of the triangular region indicating where 95% of studies should be in the absence of bias or heterogeneity) (**Figure 3**). All outcomes were downgraded for inconsistency as there was substantial heterogeneity in the meta-analysis. Overall, evidence for all outcomes was graded as low quality.

Findings

The primary focus for this review was trials including measures of all six biomarkers so that their comparative utility could be assessed (**Figure 4**). All biomarker concentrations, except lycopene, increased more from pre- to post-intervention in the arm providing higher amounts of fruit and vegetables compared to the arm providing lower amounts; α-carotene (SMD 0.63, 95% CI 0.25, 1.01), β-carotene (SMD 0.27, 95%CI 0.08, 0.45), β-cryptoxanthin (SMD 0.52, 95% CI 0.30, 0.74), lutein (SMD 0.70, 95% CI 0.37, 1.03) and vitamin C (SMD 0.94, 95% CI 0.66, 1.22). For lycopene there was no evidence of greater change in plasma concentrations (SMD -0.02, 95% CI -0.27, 0.23) in response to higher fruit and vegetable intake. There was substantial between-trial heterogeneity in the pooled effects for all biomarkers (I^2=74-94%). In the sensitivity analyses, where trials with extreme outlying results were excluded, seven out of nine trials remained in the analysis (**Supplementary figure 1**). Effect sizes were smaller for all biomarkers but a similar pattern was observed, where there were significant effects for α-carotene, β-carotene, β-cryptoxanthin, lutein and vitamin C but again no evidence of a difference for lycopene. Heterogeneity was reduced for β-crytoxanthin, lutein, lycopene and vitamin C (I^2=46-66%), but remained significant (**Supplementary figure 1**). Further sensitivity analyses utilising information for each biomarker from all available studies (indirect comparisons) (**Supplementary figure 2**) and excluding non-normally distributed data (**Supplementary figure 3**) did not alter the pattern of results.

Individual meta-analyses for each biomarker including up to nineteen trials are shown in **Supplementary figures 4-9**. For these indirect comparisons, the same pattern was observed as for direct comparisons, with statistically significant effects for all biomarkers except lycopene. For these indirect analyses we were able to additionally estimate effects for zeaxanthin (**Supplementary figure 10**) and total carotenoids (**Supplementary figure 11**), which were available in a smaller number of studies. Both showed increases in response to high compared with low amounts of fruits and vegetables but were also highly heterogeneous (I^2= 84 and 93% respectively).

All trials providing data on at least one biomarker were included in the investigation of dose response and sub-group analyses. In meta-regressions of within-trial difference in amount of fruit and vegetables (grams/day) against SMD of biomarker level, there was no evidence of a dose-response effect (all p>0.05). When the difference in the amount of fruit and vegetables consumed in each arm was categorised into portions (2-3 vs. 4-5 vs. >5 portions), a trend towards higher biomarker concentrations among trials where the group difference in fruit and vegetable intake was greater emerged but was only statistically significant for β-carotene (p=0.01, **Figure 5**).

Other notable findings from subgroup analyses included stronger effects for α-carotene, β-carotene, lutein and vitamin C in trials where participants ate meals under supervision compared to trials where all food was eaten at home, accounting for 12-38% of the heterogeneity (**Supplementary figure 12**). Shorter interventions (0-3 weeks) were associated with significantly greater effect sizes compared to longer (≥4 weeks) interventions for α- and β-carotene. There were non-significant trends for a similar effect for lutein, lycopene and vitamin C, accounting for between 6-20% of the heterogeneity (**Supplementary figure 13**). Trials in healthy populations tended to show greater effect sizes compared with trials in unhealthy populations (**Supplementary figure 14**) and this was significant for α- and β-carotene (accounting for 17-18% of the heterogeneity). In the sensitivity analysis, excluding outlying results, there was still a significant effect of disease status for α- and β-carotene. In the sensitivity meta regressions including intervention delivery, duration and participant health status all together associations were unaltered (data not shown).

Trials conducted in the USA had significantly greater effect sizes compared with those conducted in Europe for α- and β-carotene (**Supplementary figure 15**), which was robust to adjustment for other factors for α-carotene. The effect size was greater for crossover compared with parallel trials for β-carotene and lutein (**Supplementary figure 16**), which was attenuated after adjustment for other factors (data not shown). For α-carotene and lutein there was a greater effect size for trials where vegetables alone were given compared to trials where both fruit and vegetables were given (**Supplementary figure 17**), but these findings were not robust to adjustment (data not shown). There was no evidence of differences across sub-groups defined by baseline fruit and vegetable intake, fasting status, blood fraction (plasma or serum) or risk of bias (data not shown).

Discussion

In this systematic review we identified nine additional RCTs compared with a previous systematic review [14], providing the largest evidence base to date for meta-analysis of the validity of carotenoids and vitamin C based on highly controlled validation studies. While previous reviews have not been able to comment on the comparative validity of different biomarkers, our results highlight that vitamin C and 4 common carotenoids may all be equally useful as a biomarker for objectively measuring general fruit and vegetable intake.

Similar to a previous systematic review[14], vitamin C and carotenoids were identified as commonly used biomarkers for fruits and vegetables. In the previous systematic review these biomarkers are qualitatively described as consistently responding to increased fruit and vegetable intakes. Our meta-analysis provides quantitative evidence to support that vitamin C, α- and β-carotene, β-cryptoxanthin and lutein all increase in response to a high fruit and vegetable intake but high heterogeneity estimates suggest a lack of consistency in the size of the response observed between studies.

Meta-regression of fruit and vegetable dose on changes in biomarker concentration showed no evidence of a dose-response relationship for any biomarkers. While pooled biomarker

responses in sub-groups defined by increasing fruit and vegetable dose appeared to be incrementally greater, the differences were not statistically significant. The absence of dose-response in our review may be explained by ceiling effects, where plasma biomarker concentrations reach a peak and do not increase further in response to higher fruit and vegetable intakes because excess levels are stored in body tissue or excreted. In the included trials, the difference in fruit and vegetable dose was typically 5-6 portions per day, equivalent in one trial to 194 mg of vitamin C and 4 mg/day of β-carotene [29]. Vitamin C saturation can occur at intakes as low as 30-60 mg/day [40] whereas, for β-carotene, doses up to 45mg/day are within a physiologically responsive range [41]. Ceiling effects may affect vitamin C but may have less impact on the plasma response of β-carotene and other carotenoids that have a wider physiologically responsive range. However, our sub-group analyses found no evidence of differences in the pooled effects by baseline fruit and vegetable intake or baseline biomarker, even for vitamin C concentrations, indicating that ceiling effects were unlikely to be affecting dose-responses at the tested levels of intake.

Alternatively, trial integrity may have had a role masking a dose-response curve. Adherence to the intervention might be anticipated to be lower for people in groups allocated to higher doses of fruits and vegetables e.g. it's harder to comply with eating 8-9 portions per day than 4 portions per day and differential compliance by dose may explain the lack of observed dose response. Shorter (0-3 weeks) compared with longer (≥4 weeks) interventions had larger effects, which may be explained by reduced compliance in longer trials owing to intervention fatigue. The half-life of some biomarkers is relatively short, with plasma biomarker concentrations reducing to baseline over 2-3 weeks [41]. However, in this review, shorter trials were also more likely to have supervised meals. Five of eight studies of 0-3 weeks duration (63%) vs. three of eleven (27%) trials of 4+ weeks duration involved supervised meals. We found that trials with supervised meals had larger pooled effects compared with trials without supervision, likely reflecting better intervention adherence and more accurately representing the intervention-biomarker relationship.

The presence of supervised feeding in trials explained only between 12% (for α-carotene) and 38% (for lutein) of the between-trial heterogeneity, suggesting that other individual and trial-

356 level factors also influence the observed biomarker-fruit and vegetable intake relationship.
357 Individual-level factors, such as age, sex and BMI, the efficiency of absorption and excretion,
358 differences in smoking, alcohol, dietary and exercise habits and variation in the presence of
359 underlying disease/metabolic disorders, are suggested influences on the relationship between
360 fruit and vegetable intake and biomarker status [10; 41; 42]. Several of these moderating factors
361 were explored in sub-group analyses. Health status was identified as a source of
362 heterogeneity; trials that recruited participants who were overweight, hypertensive or at high
363 risk of CVD had lower pooled effect sizes than trials of healthy participants. Factors related to
364 CVD, such as chronic low grade inflammation, can affect the absorption, metabolism and
365 storage of biomarkers in the body [10], which may explain the reduced effect of interventions in
366 populations with disease/metabolic disturbances. One key trial-level difference not captured
367 fully in our sub-group analyses was the variation in the types of fruits and vegetables provided
368 to participants. Diets with fruits and vegetables that were richer in vitamin C and carotenoids
369 may have shown a stronger relationship with biomarker levels. However, although the type of
370 fruits and vegetables provided was reported in 11 out of 19 studies, the amount of each type
371 was not consistently described. Without information on both the type and amount of specific
372 fruits and vegetables it was not possible to accurately estimate the vitamin C or carotenoid
373 content of diets. We included any studies changing more than one type of fruit or vegetable in
374 order to represent 'general' changes in intake but it is possible that the micronutrient
375 composition of the fruits and vegetables provided could further explain some of the
376 heterogeneity in biomarker responses between studies.

377

378 According to the GRADE assessment, the evidence was low quality therefore “Further
379 research is very likely to have an important impact on our confidence in the estimate of effect
380 and any estimate of effect is very uncertain” [20]. The interpretation of results in this review is
381 limited by the high level of heterogeneity observed between trials, which could not be fully
382 explained in sub-group analyses. In assessing fruit and vegetable intake not only is there
383 likely to be large between-population variation, but there is also likely to be large variation in
384 the biomarker response of individuals [41; 42; 43]. The evidence from this meta-analysis does not
385 provide support for the use of biomarkers to estimate absolute levels of fruit and vegetable
386 intake because of a lack of dose-response effect. It also does not provide support for
387 estimating changes in fruit and vegetable intake in individuals because only group-level

differences were quantified in the trials. Further studies of the determinants of within and between individual variation in vitamin C and carotenoid levels in large-scale studies with biomarkers measured at multiple time-points will help to understand the relative importance of changes in fruit and vegetable consumption for changes in biomarker concentrations.

Strengths of the present systematic review include the identification of nine trials additional to the previous review, thus allowing an in-depth exploration of between-trial heterogeneity and a comparative analysis restricted to nine trials with a common set of biomarkers measured (five were newly identified by our update to the review). However, some uncertainty remains regarding the comparative utility of different biomarkers. Although vitamin C had the greatest response, it was not significantly greater from the response of other biomarkers. Therefore, no particular biomarker can be recommended above the others on the basis of our results thus selection may be based on study needs. The review included only randomised controlled trials that directly observed or provided fruit and vegetables. This restriction reduced the number of included trials compared to previous reviews [14], but is considered a strength because observed effects are less confounded by potential exposure misclassification related to low compliance or other dietary changes associated with dietary interventions.

The present systematic review and meta-analysis confirm that vitamin C and carotenoids (except lycopene) are responsive to changes in general fruit and vegetable intake at a group level. However, the evidence was of low quality, there was no clear evidence of dose-response or that any single biomarker was more responsive. Further work is required to understand the determinants of biomarker variation among individuals.

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541 Table 1: Characteristics of 19 randomised controlled trials of fruit and vegetable intake on biomarker concentrations

Author	Year	Population	Location	Design	N	Mean age (yrs)	Method	Intervention	Intervention duration (wks)	Run-in (wks)	Fruit and vegetable intake (Portions/day)		Blood fraction	Fasted state	Smokers excluded?
											Baseline	Treatment			
Baldrick [26]	2012	COPD	UK	Parallel	81	62	Provided F&V, delivered to homes	F&V	13	0	GP_1 1.4 GP_2 1.5	GP_1 1.9 GP_2 6.1	Plasma	Non-fasting	No
Berry [18]	2010	Elevated blood pressure	UK	Cross over	57	45	Provided F&V, delivered to homes	F&V	6	3	GP_1 3.6 GP_2 3.6 GP_3 3.6	GP_1 3.6 GP_2 6.7 GP_3 8.0	Plasma	Fasted	Yes
Brevik [34]	2004	Healthy - students	Norway	Cross over	39	23	Foods supplied and eaten under supervision	F&V	2	1	GP_1 NR GP_2 NR	GP_1 3.8 GP_2 9.4	Plasma	Fasted	Yes
Briviba [27]	2008	Healthy - general	Germany	Parallel	63	NR	Foods supplied and lunch eaten under supervision	F&V	3	1	GP_1 2.8 GP_2 3.3 GP_3 3.1	GP_1 2.5 GP_2 5.8 GP_3 9.8	Plasma	NR	Yes
Broekmans [28]	2000	Healthy - Low F&V	The Netherlands	Parallel	48	49	Foods supplied and dinner eaten under supervision	F&V	4	NR	GP_1 2.0 GP_2 2.0	GP_1 1.3 GP_2 6.3	Plasma	Fasted	No
Chong [29]	2013	Increased	UK	Parallel	221	51	Provided F&V,	F&V	18	2	GP_1 3.9	GP_1 4.5	Plasma	Fasted	No

		CVD risk				delivered to homes				GP_2 3.8 GP_3 3.4	GP_2 7.6 GP_3 8.1		
Crane [16]	2011	Overweight (BMI 25-45), post-menopausal women	USA	Cross over	50	59	Provided F&V, delivered to homes	Veg only	3	4	GP_1 NR GP_2 NR GP_3 NR	GP_1 1.6 GP_2 3.6 GP_3 7.7	Plasma	Fasted	No
Dragsted [17]	2004	Healthy – general	Denmark	Parallel	48	26	Foods supplied and lunch eaten under supervision	F&V	4	0.4	GP_1 3.3 GP_2 4.1	GP_1 3.3 GP_2 7.5	Plasma	Fasted	Yes
Gill [23]	2004	Healthy volunteers	UK	Parallel	20	26	Foods provided (NR where consumed)	Veg only	2	1	GP_1 NR GP_2 NR	GP_1 0.0 GP_2 1.4	Plasma	Fasted	NR
Howe [39]	2009	Obese	USA	Parallel	37	33	Food provided at breakfast and lunch	F&V	13	NR	GP_1 NR GP_2 NR	GP_1 1.2 GP_2 2.5	Serum	Fasted	No
Martini [38]	1995	Healthy	USA	Crosso ver	23	26	Ate on site or picked up to eat at home	Veg only	1	0.7	GP_1 NR GP_2 NR GP_3 NR	GP_1 0.0 GP_2 6.8 GP_3 8.6	Plasma	Fasted	Yes
McCall [30]	2009	Hypertensio n	UK	Parallel	147	52	Food delivered to home, weekly phone calls	F&V	8	4	GP_1 0.9 GP_2 1.1 GP_3 1.1	GP_1 1.1 GP_2 3.2 GP_3 5.6	Serum	Fasted	No

Moller [35]	2003	Healthy - general	Denmark	Parallel	48	26	Foods supplied and lunch eaten under supervision	F&V	4	0.6	GP_1 3.3 GP_2 4.2	GP_1 0.0 GP_2 7.5	Plasma	Fasted	Yes
Neville [31]	2013	Healthy, Older adults	UK	Parallel	83	71	Advice and home deliveries of F&V	F&V	16	0	GP_1 1.4 GP_2 1.4	GP_1 1.8 GP_2 6.0	Plasma	Fasted	No
Rantala [15]	2002	Healthy women	Finland	Crossover	37	43	Ate on site or picked up to eat at home	F&V	5	2	GP_1 NR GP_2 NR	GP_1 2.9 GP_2 8.3	Plasma	Fasted	Yes
Thompson [36]	2005	Healthy - women's health interest group	USA	Parallel	246	48	Cookbook with daily menus and recipes and one-third of meals supplied	F&V	4	2	GP_1 4.5 GP_2 4.5	GP_1 5.4 GP_2 13.8	Plasma	NR	Yes
Thompson [37]	2005 b	Healthy - unclear source	USA	Parallel	64	49	Foods prescribed	F&V	2	0	GP_1 NR GP_2 NR	GP_1 5.4 GP_2 18.2	Plasma	Non-fasting	NR
Van het Hof [32]	1999	Healthy - general	The Netherlands	Parallel	55	22	Foods supplied (90% of energy intake) and partially eaten under supervision	Veg only	4	NR	GP_1 NR GP_2 NR	GP_1 1.6 GP_2 6.1	Plasma	Fasted	Yes

Wallace [33]	2013	High CVD risk	UK	Parallel	105	56	Advice plus weekly home deliveries of F&V telephone call from researcher weekly	F&V	12	4	GP_1 1.7 GP_2 1.7 GP_3 1.6	GP_1 1.8 GP_2 3.8 GP_3 7.1	Plasma	Fasted	No

BMI, Body Mass Index; COPD, Chronic Obstructive pulmonary Disease; CVD, Cardiovascular disease; F&V, Fruit and vegetables; GP, Group; NR, Not reported

542

Figure Legends

Figure 1: PRISMA diagram of search results

Figure 2: Summary of risk of bias among the 9 studies with 6 biomarkers measured.

Figure 3: Funnel plots of 9 randomised controlled trials of different doses of fruit and vegetable intake on biomarker concentrations

Figure 4: Summary of pooled difference between arms consuming higher vs. lower amounts of fruit and vegetables for standardised mean change (SMC) of biomarkers from pre- to post-intervention in trials with all 6 biomarkers measured. SMC represents a standard deviation of pre-intervention biomarker levels within each study. I^2 is an indicator of between-trial heterogeneity. Random effects meta-analysis was used to pool mean differences*. Includes the following studies for ALL biomarkers: Baldrick[26]; Briviba[27]; Broekmans[28]; Chong[29]; Gill[23]; McCall[30]; Neville[31]; Van Het Hof[32]; Wallace[33]. Total number of trials is 9; total number of arms being compared is 22; total number of people included is 667.*

Figure 5: Summary of pooled differences between arms consuming higher vs. lower amounts of fruit and vegetables in standardised mean change (SMC) of biomarkers from pre- to post-intervention in all trials with available data grouped by amount of fruit and vegetables provided during the intervention. SMC represents a standard deviation of pre-intervention biomarker levels within each study. I^2 is an indicator of between-trial heterogeneity. Random effects meta-analysis was used to pool mean differences. P value is from meta-regression test for trend across categories. *Includes all studies up to n=19 based on availability of biomarker in each study.*

Figure 1

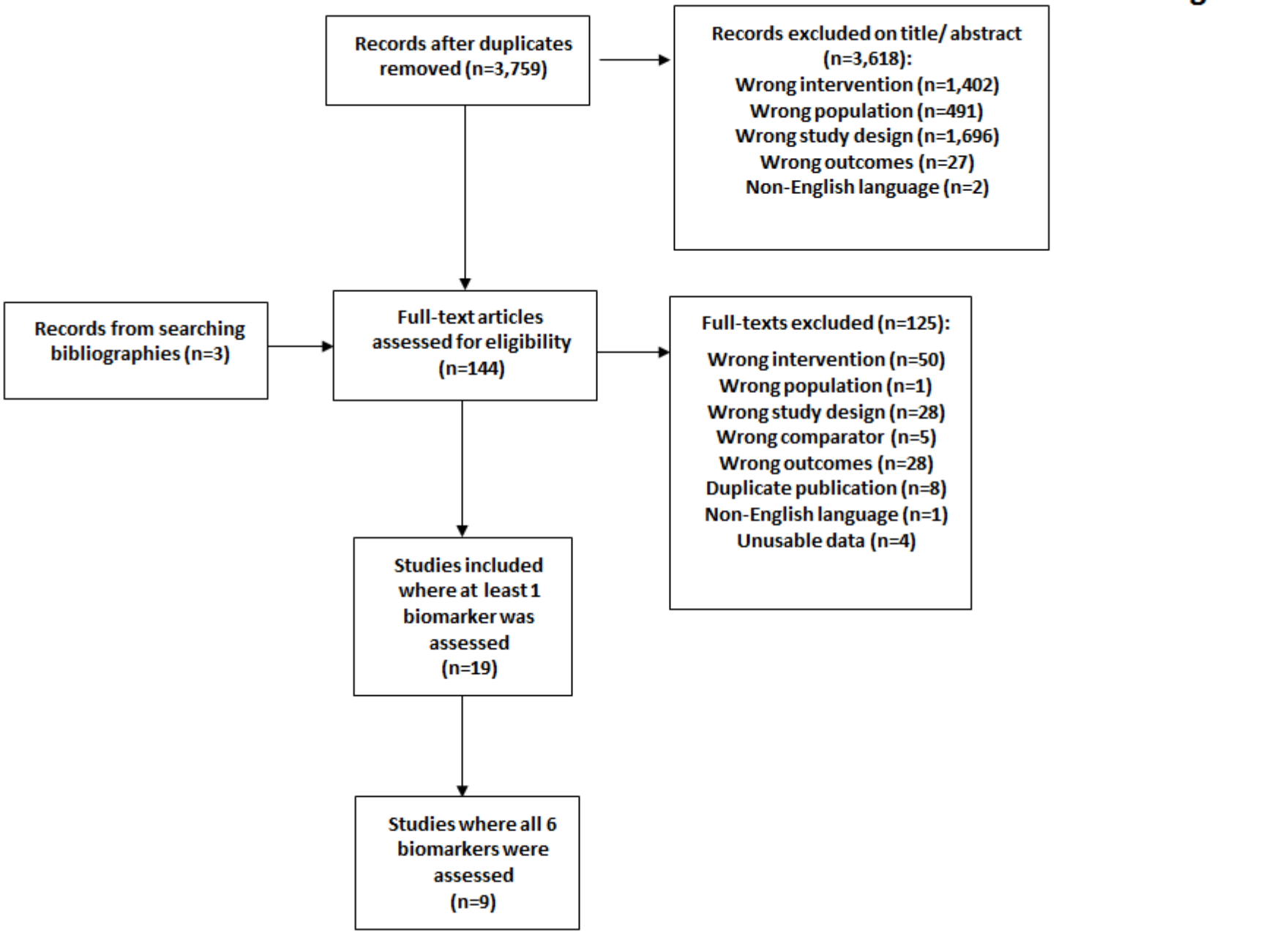

Records after duplicates removed (n=3,759)
Records excluded on title/ abstract (n=3,618):
Wrong intervention (n=1,402)
Wrong population (n=491)
Wrong study design (n=1,696)
Wrong outcomes (n=27)
Non-English language (n=2)
Records from searching bibliographies (n=3)
Full-text articles assessed for eligibility (n=144)
Full-texts excluded (n=125):
Wrong intervention (n=50)
Wrong population (n=1)
Wrong study design (n=28)
Wrong comparator (n=5)
Wrong outcomes (n=28)
Duplicate publication (n=8)
Non-English language (n=1)
Unusable data (n=4)
Studies included where at least 1 biomarker was assessed (n=19)
Studies where all 6 biomarkers were assessed (n=9)

Figure 2

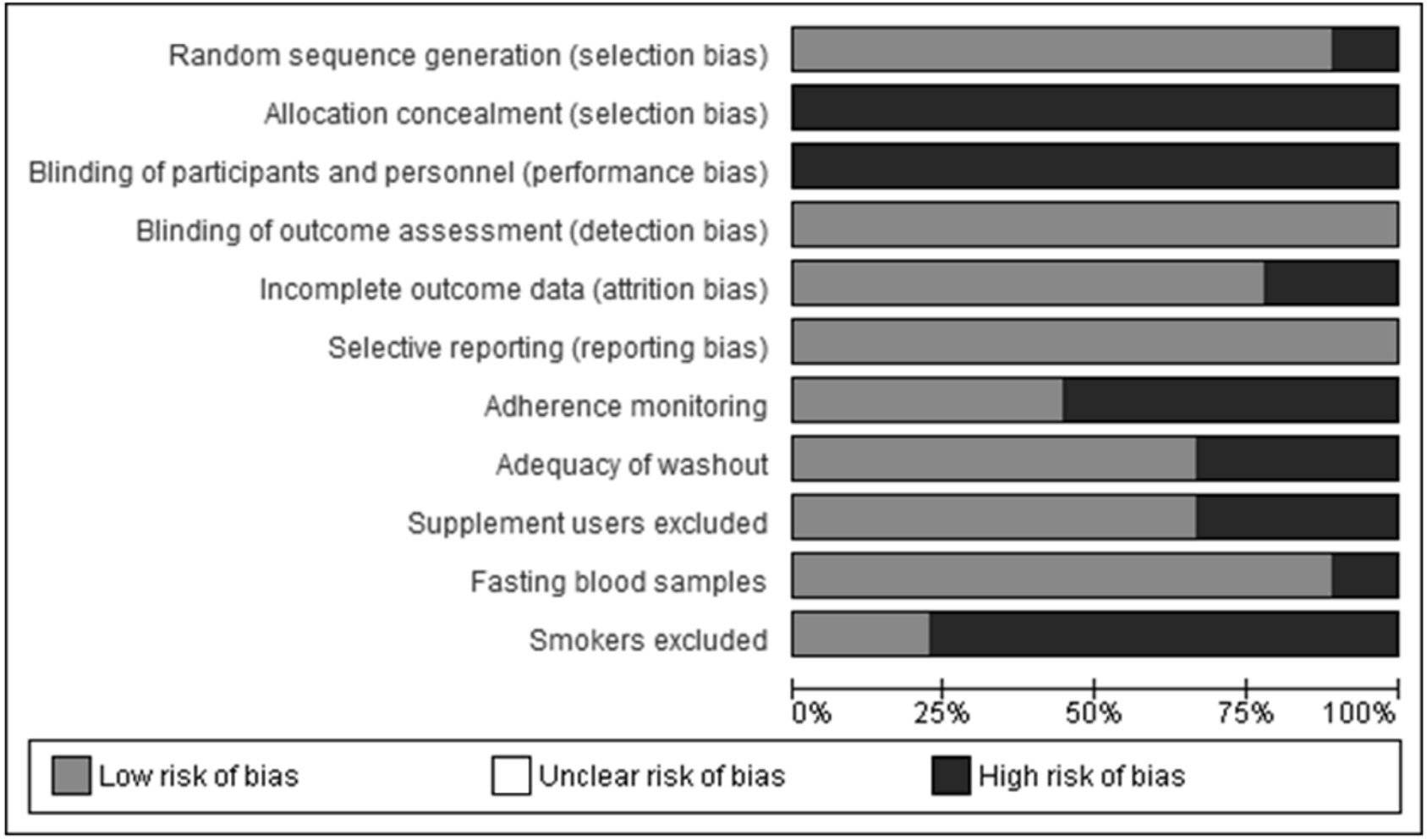
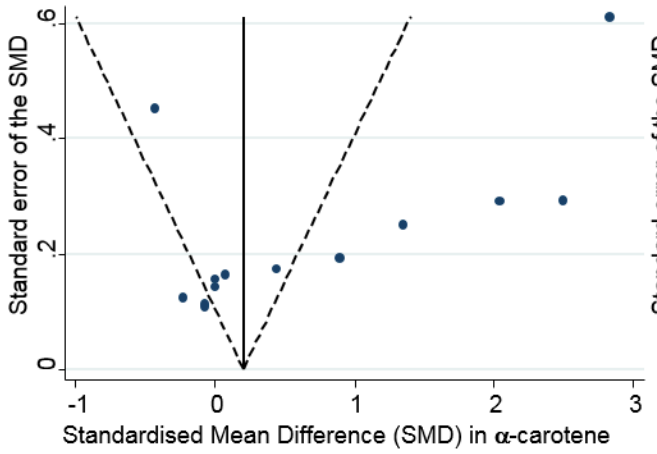
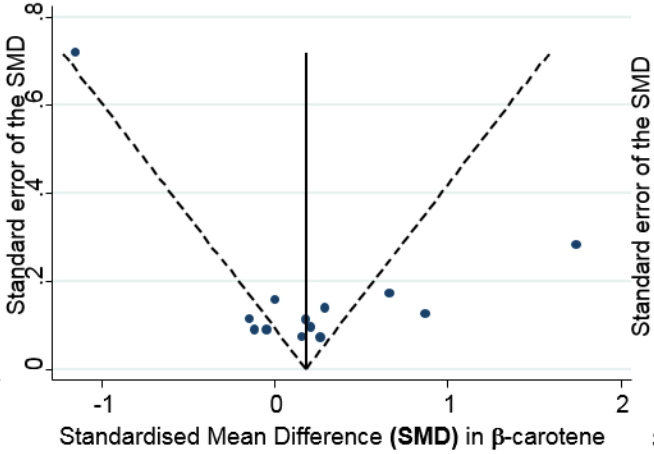

Random sequence generation (selection bias)
Allocation concealment (selection bias)
Blinding of participants and personnel (performance bias)
Blinding of outcome assessment (detection bias)
Incomplete outcome data (attrition bias)
Selective reporting (reporting bias)
Adherence monitoring
Adequacy of washout
Supplement users excluded
Fasting blood samples
Smokers excluded
0%
25%
50%
75%
100%
Low risk of bias
Unclear risk of bias
High risk of bias

Figure 3


Standard error of the SMD
0
.2
.4
.6
-1
0
1
2
3
Standardised Mean Difference (SMD) in α-carotene


Standard error of the SMD
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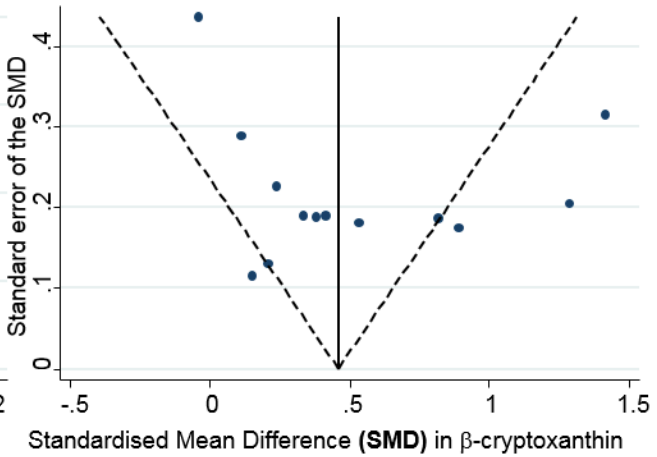
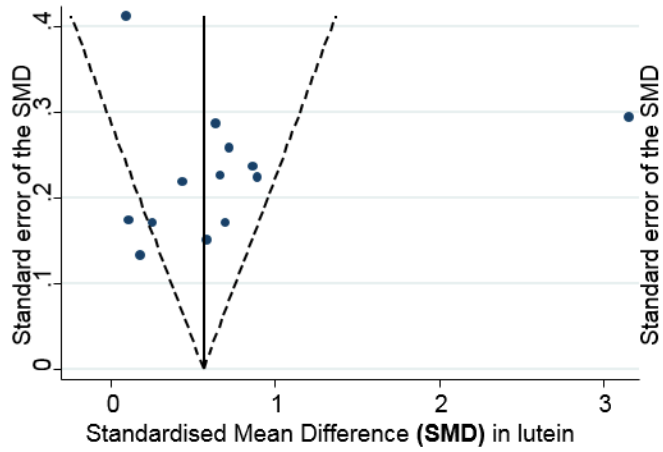
.4
.6
.8
-1
0
1
2
Standardised Mean Difference **(SMD)** in β-carotene


Standard error of the SMD
0
.1
.2
.3
.4
-.5
0
.5
1
1.5
Standardised Mean Difference **(SMD)** in β-cryptoxanthin


Standard error of the SMD
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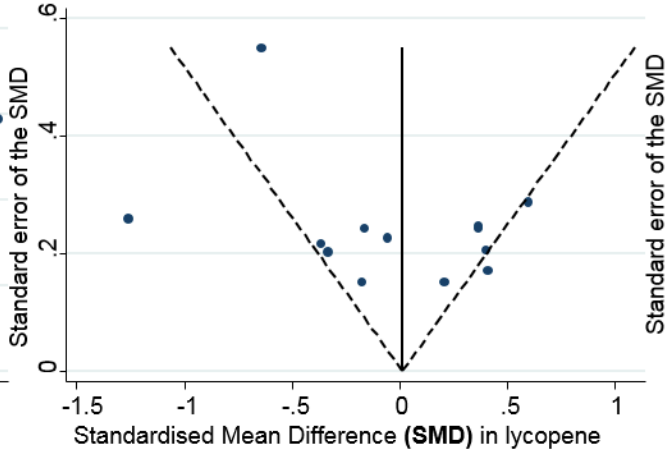
.1
.2
.3
.4
0
1
2
3
Standardised Mean Difference **(SMD)** in lutein


Standard error of the SMD
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.4
.6
-1.5
-1
-.5
0
.5
1
Standardised Mean Difference **(SMD)** in lycopene

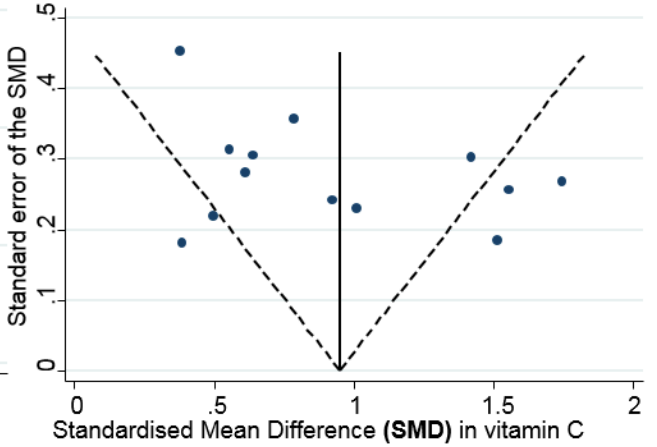

Standard error of the SMD
0
.1
.2
.3
.4
.5
0
.5
1
1.5
2
Standardised Mean Difference **(SMD)** in vitamin C

Figure 4

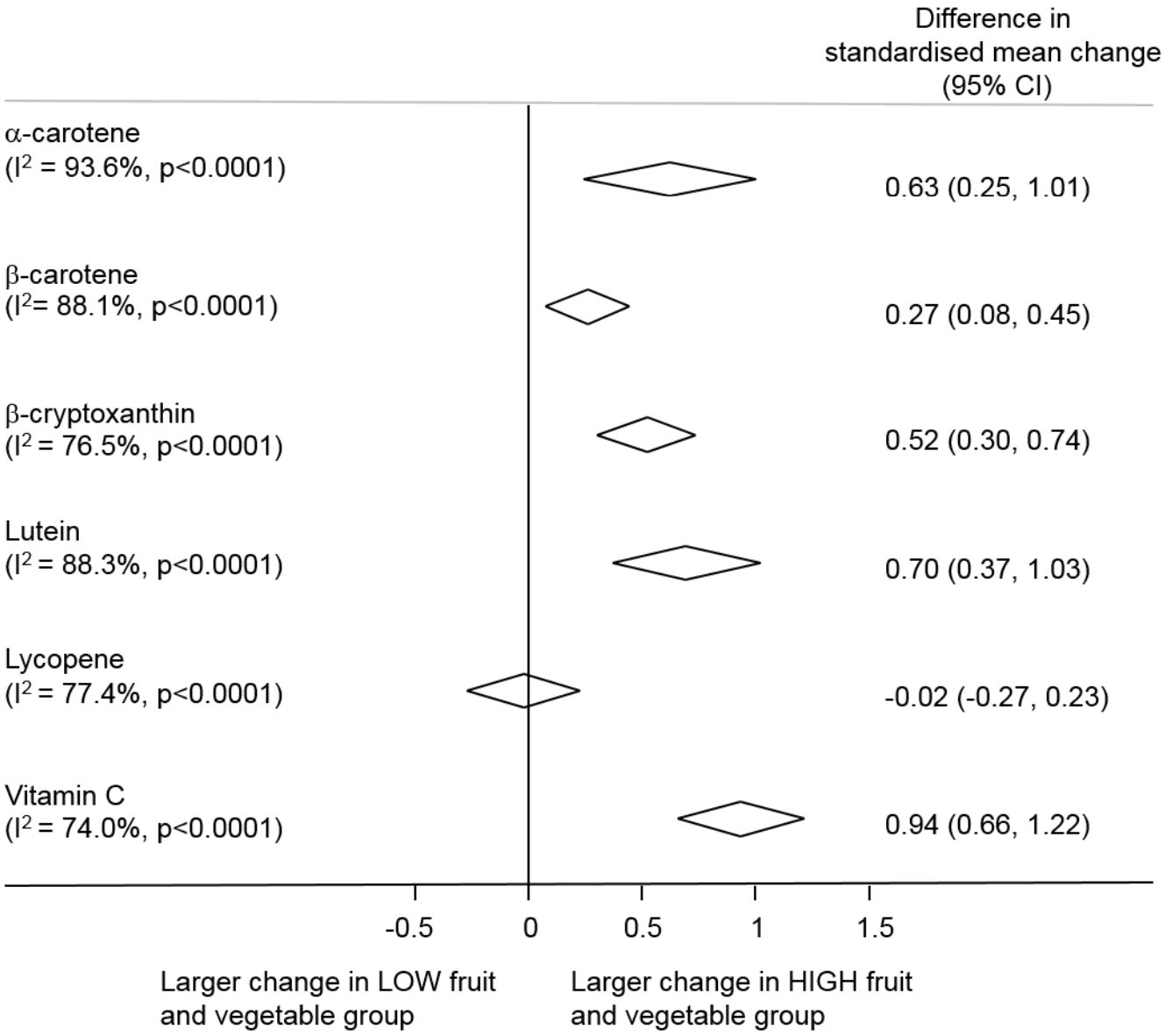
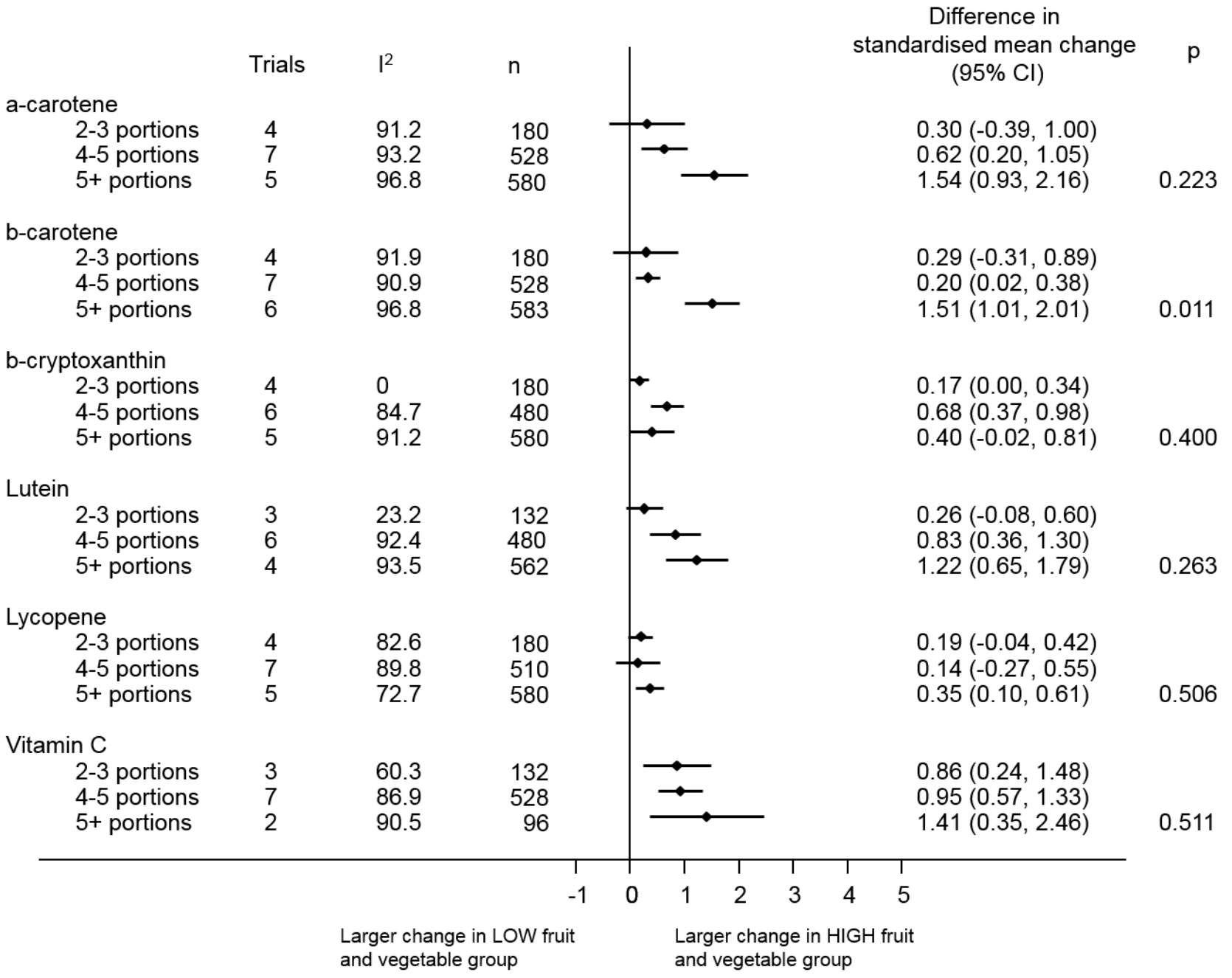

Difference in standardised mean change (95% CI)
α-carotene
(I² = 93.6%, p<0.0001)
0.63 (0.25, 1.01)
β-carotene
(I²= 88.1%, p<0.0001)
0.27 (0.08, 0.45)
β-cryptoxanthin
(I² = 76.5%, p<0.0001)
0.52 (0.30, 0.74)
Lutein
(I² = 88.3%, p<0.0001)
0.70 (0.37, 1.03)
Lycopene
(I² = 77.4%, p<0.0001)
-0.02 (-0.27, 0.23)
Vitamin C
(I² = 74.0%, p<0.0001)
0.94 (0.66, 1.22)
-0.5
0
0.5
1
1.5
Larger change in LOW fruit and vegetable group
Larger change in HIGH fruit and vegetable group

Figure 5


Trials
I²
n
Difference in standardised mean change (95% CI)
p
a-carotene
2-3 portions 4 91.2 180 0.30 (-0.39, 1.00)
4-5 portions 7 93.2 528 0.62 (0.20, 1.05)
5+ portions 5 96.8 580 1.54 (0.93, 2.16) 0.223
b-carotene
2-3 portions 4 91.9 180 0.29 (-0.31, 0.89)
4-5 portions 7 90.9 528 0.20 (0.02, 0.38)
5+ portions 6 96.8 583 1.51 (1.01, 2.01) 0.011
b-cryptoxanthin
2-3 portions 4 0 180 0.17 (0.00, 0.34)
4-5 portions 6 84.7 480 0.68 (0.37, 0.98)
5+ portions 5 91.2 580 0.40 (-0.02, 0.81) 0.400
Lutein
2-3 portions 3 23.2 132 0.26 (-0.08, 0.60)
4-5 portions 6 92.4 480 0.83 (0.36, 1.30)
5+ portions 4 93.5 562 1.22 (0.65, 1.79) 0.263
Lycopene
2-3 portions 4 82.6 180 0.19 (-0.04, 0.42)
4-5 portions 7 89.8 510 0.14 (-0.27, 0.55)
5+ portions 5 72.7 580 0.35 (0.10, 0.61) 0.506
Vitamin C
2-3 portions 3 60.3 132 0.86 (0.24, 1.48)
4-5 portions 7 86.9 528 0.95 (0.57, 1.33)
5+ portions 2 90.5 96 1.41 (0.35, 2.46) 0.511
-1 0 1 2 3 4 5
Larger change in LOW fruit and vegetable group
Larger change in HIGH fruit and vegetable group